REMARKS

With entry of the amendment, claims 1-55 are pending in the application. Claims 30, 35-47, 52, and 55 are withdrawn from consideration. Claims 1-5, 7, 9-19, 21, 24-28, 50 and 53 are rejected, and claims 6, 20, 22, 23, 31-34, 48, 51, and 54 are objected to.

In view of the amendments above and the arguments below, Applicants respectfully request reconsideration on the merits of the application and allowance of the claims.

Allowable claims

The Examiner has indicated that claims 6, 20, 22, 23, 31-34, 48, 51, and 54 would be allowable if rewritten in independent form and limited to MONO-15. Claim 29 would be allowable if limited to MONO-15. Applicants believe that, based on the Examiner's indicating that claims 23 and 29 are allowable, claim 30 should be allowable.

Applicants agree that the instant application is the first report of using MONO-15 to analyze microsatellite instability. However, patentability does not turn solely on the use of MONO-15, and the instant claims need not be limited to MONO-15 in order to be patentable over the prior art.

Rejections under 35 U.S.C. 112, second paragraph

Claim 22 depends from claim 16, which requires "a set of at least three loci". Claim 22 recites "the set of at least two microsatellite loci", for which there is no antecedent basis. Applicants have amended claim 22 for reasons unrelated to patentability to clarify that the "the set of at least three microsatellite loci" is a set of at least nine loci.

Rejections under 35 U.S.C. 101 double patenting

Claims 1-3, 7, 9-15, 50, and 53 are provisionally rejected under 35 U.S.C. 101 as claiming the same invention as that of claims 1, 41-45, 47, 48, and 51-56 of copending Application No. 09/841,366. Applicants will cancel the corresponding claims in the related application upon allowance of the claims in the instant application.

Rejections under 35 U.S.C. 102(b)

Claims 1-5, 9-12, 14-19, 21, 24-26, 28, 50, and 53 are rejected under 35 U.S.C. 102(b) as being anticipated by Frazier et al. (Oncology Reports 6:497-505, 1999). Frazier et al. is characterized as teaching "a method for analyzing micro-satellite loci (five loci)", which the

Applicants disagree with the Examiner's characterization of Frazier et al. as teaching co-amplification. There is nothing in Frazier et al. to suggest co-amplification of at least one mononucleotide repeat loci and at least two tetranucleotide repeat loci in a multiplex amplification reaction, as required by claim 1. Frazier et al. discusses detection of MSI with radiolabeled PCR products, beginning at page 499. One of skill in the art would appreciate that Frazier et al. did not perform co-amplification of any combination of loci in a multiplex amplification reaction, let alone at least one mononucleotide repeat locus and at least two tetranucleotide repeat loci. Autoradiograms of electrophoretically separated, radiolabeled PCR products presented on page 501, Fig. 2 (labeled "Representative screening of some of the microsatellites used in this study"), clearly show that the five loci (D17S250, VWF, BAT26, FGA, and BAT25) were not co-amplified, but rather, were amplified and analyzed separately.

Furthermore, the Frazier et al. description of detection of MSI with fluroescently labeled PCR products (p. 500, second column; p. 502, Fig. 3) clearly indicates Frazier et al. did not co-amplify the microsatellite loci in a multiplex amplification reaction, because each marker was run in a separate lane. As one skilled in the art would appreciate, had the loci been co-amplified, one could not simply run each marker in a separate lane.

Frazier et al. does not teach all of the limitations of claim 3, which requires co-amplification of at least three microsatellite loci comprising at least two tetranucleotide repeat loci selected from the group consisting of FGA, D1S518, D1S547, D1S1677, D2S1790, D3S2432, D5S818, D5S2849, D6S1053, D7S3046, D7S1808, D7S3070, D8S1179, D9S2169, D10S1426, D10S2470, D12S391, D17S1294, D17S1299, and D18S51. Of the tetranucleotide repeat loci recited in claim 3, Frazier et al. discloses only one locus (FGA), and the method of the present invention requires co-amplification of at least two of the recited tetranucleotide repeat loci.

Claim 5 requires co-amplification of at least five microsatellite loci, wherein at least two loci are selected from BAT-25, BAT-26, MONO-11, and MONO-15, and at least three tetranucleotide loci are selected from FGA, D1S518, D1S547, D1S1677, D2S1790, D3S2432, D5S818, D5S2849, D6S1053, D7S3046, D7S1808, D7S3070, D8S1179, D9S2169, D10S1426, D10S2470, D12S391, D17S1294, D17S1299, and D18S51. Although Frazier et al. discloses two mononucleotide repeat loci (BAT-25 and BAT-26) and the tetranucleotide FGA, it does not teach any of the remaining loci recited, let alone co-amplification of at least five microsatellite loci, including at least three of the recited tetranucleotide loci.

Like claim 1, claim 15 requires co-amplification of at least one mononucleotide repeat loci

any of the remaining loci recited, let alone co-amplification of at least five microsatellite loci, including at least three of the recited tetranucleotide loci.

Like claim 1, claim 15 requires co-amplification of at least one mononucleotide repeat loci and at least two tetranucleotide repeat loci. As discussed above, Frazier simply does not teach co-amplification of at least one mononucleotide repeat locus and at least two tetranucleotide repeat loci.

Claim 17 requires co-amplification of at least three microsatellite loci comprising, comprising at least two tetranucleotide repeat loci selected from the group consisting of FGA, D1S518, D1S547, D1S1677, D2S1790, D3S2432, D5S818, D5S2849, D6S1053, D7S3046, D7S1808, D7S3070, D8S1179, D9S2169, D10S1426, D10S2470, D12S391, D17S1294, D17S1299, and D18S51. Of the tetranucleotide repeat loci recited in claim 17, Frazier et al. discloses only one locus (FGA), and the method of claim 17 requires co-amplification of at least two of the specifically recited tetranucleotide repeat loci.

Claim 19 requires co-amplification of at least five microsatellite loci, wherein at least two loci are selected from BAT-25, BAT-26, MONO-11, and MONO-15, and at least three tetranucleotide loci are selected from FGA, D1S518, D1S547, D1S1677, D2S1790, D3S2432, D5S818, D5S2849, D6S1053, D7S3046, D7S1808, D7S3070, D8S1179, D9S2169, D10S1426, D10S2470, D12S391, D17S1294, D17S1299, and D18S51. Although Frazier et al. discloses two of the mononucleotide loci (BAT-25 and BAT-26), as well one tetranucleotide repeat locus (FGA) it does not teach any of the remaining loci, let alone co-amplification of at least five microsatellite loci, including at least three of the recited tetranucleotide loci.

Claim 50 requires co-amplification of at least three microsatellite loci, wherein at least one mononucleotide locus is selected from BAT-25, BAT-26, MONO-11, and MONO-15, and at least two tetranucleotide loci are selected from FGA, D1S518, D1S547, D1S1677, D2S1790, D3S2432, D5S818, D5S2849, D6S1053, D7S3046, D7S1808, D7S3070, D8S1179, D9S2169, D10S1426, D10S2470, D12S391, D17S1294, D17S1299, and D18S51. Although Frazier et al. discloses two of the mononucleotide loci (BAT-25 and BAT-26), as well one tetranucleotide repeat locus (FGA), it does not teach any of the remaining loci, let alone co-amplification of at least three microsatellite loci, including at least two of the recited tetranucleotide loci.

Because Frazier et al. does not teach all of the claim limitations, rejection of the claims under 35 U.S.C. 102(b) as being anticipated by Frazier et al. is improper. Accordingly, Applicants respectfully request that the Examiner withdraw the rejection.

Rejection of claims under 35 U.S.C. 102(e)

Claims 1-5, 10-19, 24-28, 50, and 53 are rejected under 35 U.S.C. 102(e) as being anticipated by Ruschoff et al. U. S. Patent No. 6,150,100.

Ruschoff et al. is cited as teaching multiplex analysis (column 5, lines 14-19, column 6, lines 49-50) and providing primers for microsatellite loci comprising mono-, di-, tetra- and pentanucleotide repeat loci.

Applicants respectfully disagree with the Examiner's interpretation of Ruschoff. Claim 1 requires co-amplification of at least one mononucleotide repeat locus and at least two tetranucleotide repeat loci. At column 5, lines 14-19 of Ruschoff et al., it is stated, "As an alternative 2 or several loci were also analyzed together in one reaction mixture in a duplex or multiplex analysis if fragment sizes were expected which differ considerably from one another." At column 6, lines 33-43, Ruschoff discusses analyzing microsatellite instability of various loci, including mononucleotide repeat loci (BAT25, BAT26, and BAT40) and tetranucleotide repeat loci (HPRT, MYCL1, and RB).

In Ruschoff et al., examples of individually amplified alleles of all 25 examined gene loci are shown in Fig. 2A, whereas only a single example of a duplex analysis (BAT40 and the dinucleotide D2S1283) is shown in Fig. 2B. Applicants respectfully submit that a single example showing co-amplification of a mononucleotide repeat locus and a dinucleotide repeat locus as shown in Fig. 2B does not teach co-amplification of at least one mononucleotide repeat locus and at least two tetranucleotide repeat loci, as required by claims 1, 15, and 53, or by claims dependent therefrom, let alone co-amplification of at least five microsatellite loci as required by claims 5 and 19. Nor does Ruschoff teach any of the particular tetranucleotide repeat loci recited claims 3, 5, 16, 19, or 53, let alone co-amplification of at least two of the specifically recited tetranucleotide repeat loci with at least one mononucleotide repeat locus.

In fact, Ruschoff teaches away from using tetranucleotide repeat loci in MSI analysis. As can be seen from Fig. 3, two of the three tested tetranucleotide repeat loci (i.e., HPRT and RB) were relatively insensitive to identifying microsatellite instability in RER+ tumors, whereas one of the tetranucleotide repeat loci (MYCL1) showed microsatellite instability with RER- tumors. Beginning at column 7, line 55 of Ruschoff, problems associated selecting loci for analyzing microsatellite instability were discussed:

> *certain loci are specifically and regularly affected by MIN in RER+ tumors. In this respect a uniform result for MIS was only achieved with mononucleotide repeats (BAT25, BAT26, BAT40). Each of these loci was altered in the same 5 RER+ tumours (Nos. 1, 2, 8, 13, 16) but none had MIN in RER- tumours or "low MIN" tumours. In contrast all other*

tested loci except for Mfd15 were either mutated less often in the RER+ tumours or additionally altered in "low MIN" tumours. For example in the case of the APC locus it was possible to detect MIS not only in all RER+ tumours but also in tumour No. 20. Five loci exhibited MINs in 4/6 RER+ tumours but only D2S123 was unaltered in non-RER+ tumours. In contrast locus MYCL1, which was also altered in 4/6 RER+ tumours, exhibited additional instabilities in 3 "low MIN" tumours so that this locus is for example unsuitable as a marker.

In other words, Ruschoff taught the unsuitability of tetranucleotide repeat loci for use in microsatellite instability analysis because they were either unable to detect RER+ tumors or gave false positives with RER- tumors.

Because Ruschoff does not teach all of the claim limitations, the rejection under 35 U.S.C. 102(e) is improper. Applicants respectfully request that the rejection be withdrawn.

Rejections under 35 U.S.C. 103(a)

In the previous Office Action, claims 1, 2, 9-16, 21, 24-28, and 31-34 were rejected under 35 U.S.C. 103(a) as being unpatentable over Ruschoff et al. (U.S. Patent No. 6,150,100) in view of Schumm et al. (U.S. Patent No. 5,843,660), and claims 4-8, 18-20, 22, 23, 29, and 30 were rejected under 35 U.S.C. 103(a) as being unpatentable over Ruschoff et al. (U.S. Patent No. 6,150,100) in view of Schumm et al. (U.S. Patent No. 5,843,660), further in view of Kieback (U.S. Patent No. 5,645,995) and Sulston et al. (Genome Res. 8:1097-1108, sequence alignment from GenEmbl).

In the second non-final Office Action, the Examiner indicated that Applicant's arguments made in the previous response were persuasive with respect to the rejection of claims 6-8, 20, 22, 23, 29, and 31-34. However, Applicants' argument that Ruschoff et al. does not teach 'analyzing or detecting microsatellite instability comprising at least three microsatellite loci comprising at least one mono and at least two tetranucleotide repeat loci' was not deemed persuasive because "Ruschoff et al. did teach duplex or multiplex analysis and co-amplification of 25 loci which include the limitation of at least one mononucleotide repeat locus and at least two tetranucleotide repeat loci, as claimed in claims 1, 5, and 15." Accordingly, the Examiner apparently maintained the rejection of claims 1, 2, 4, 5, 9-16, 18, 19, 21, 24-28, and 30 under 103(a) for reasons set forth previously.

The Examiner noted in the previous Office Action that Ruschoff et al. does not teach a method of analyzing or detecting microsatellite instability comprising co-amplifying at least three microsatellite loci comprising at least one mononucleotide repeat locus and at least two

tetranucleotide loci, as required by claims the claims. However, in the second non-final Office Action, the Examiner stated that Ruschoff et al. teaches duplex amplification (citing column 5, lines 14-19).

Applicants submit that nothing in Ruschoff et al. nor Schumm et al. provide the suggestion or motivation to modify or combine reference teachings to make a method of analyzing microsatellite loci comprising co-amplifying at least one mononucleotide repeat locus and at least two tetranucleotide repeat loci, as required by claims 1 and 15.

Applicants have reviewed the Ruschoff et al. publication and identified only a single example of a duplex amplification reaction involving one mononucleotide repeat loci and one dinucleotide repeat loci. There is absolutely no teaching or suggestion to co-amplify at least one mononucleotide repeat locus and at least two tetranucleotide repeat loci, as required by claims 1 and 15, and all claims dependent therefrom.

Applicants respectfully submit that the Examiner erred in concluding that Ruschoff et al. teaches co-amplification of 25 loci which includes the limitation of at least one mononucleotide repeat locus and at least two tetranucleotide repeat loci. As pointed out above in Applicants' response to the rejection of claims under 35 U.S.C. 102(e), Ruschoff et al. teaches individually amplifying each of 25 loci in separate PCR reactions (Fig. 2A). The only co-amplification exemplified included a mononucleotide repeat locus (BAT40) and a dinucleotide repeat locus (D2S1283).

The only guidance that Ruschoff et al. provides with respect to co-amplifying the 25 loci of Ruschoff et al. is that "two or several loci were also analyzed together in one reaction mixture in a duplex or multiplex analysis if fragment sizes were expected which differ considerably from one another." The teachings of Ruschoff would not suggest to one skilled in the art the desirability of using at least two tetranucleotide repeat loci in a multiplex reaction. In fact, the ordinary practitioner wishing to reliably detect microsatellite instability using a limited number of PCR reactions would not be motivated to modify Ruschoff *et al.* to co-amplify at least one mononucleotide repeat locus and at least two tetranucleotide repeat loci. As discussed above, Ruschoff teaches away from using tetranucleotide repeat loci in MSI analysis. Ruschoff teaches that tetranucleotide repeat loci are relatively insensitive for identifying microsatellite instability in RER+ tumors, and at the same time, give false positives with RER- tumors. Ruschoff concluded that the only tetranucleotide locus with sufficient sensitivity to identify RER+ tumors exhibited additional instabilities in 3 "low MIN" tumours and was, therefore, unsuitable as a marker.

Schumm et al. teaches co-amplifying short tandem repeat (STR) loci, which are defined as

regions of the human genome containing short repetitive sequence elements of 3 to 7 bases pairs in length (Schumm et al., column 12, lines 27-29). In contrast, the methods of claims 1, 2, 4, 9-16, 18, 19, 21, 24-28, and 30 of the subject application require co-amplifying at least three microsatellite loci comprising at least one mononucleotide repeat locus and at least two tetranucleotide repeat loci.

The art of record does not combine to teach or suggest co-amplifying at least one mononucleotide repeat locus and at least two tetranucleotide repeat loci selected from FGA, D1S518, D1S1677, D2S1790, D3S2432, D5S818, D5S2849, D6S1053, D7S3046, D7S1808, D8S1179, D9S2169, D10S1426, D10S2470, D12S391, D17S1294, D17S1299, and D18S51, in a multiplex reaction, as required by claims 3 and 17.

The art of record does not combine to teach or suggest analyzing at least one of BAT-25, BAT-26, MONO-11, and MONO-15 co-amplified with at least two tetranucleotide loci in a multiplex reaction, as required by claims 4 and 18.

The art of record does not combine to teach or suggest analyzing at least five microsatellite loci co-amplified in a multiplex comprising at least two mononucleotide repeat loci selected from one of BAT-25, BAT-26, MONO-11, and MONO-15 and at least with at least three tetranucleotide loci selected from FGA, D1S518, D1S1677, D2S1790, D3S2432, D5S818, D5S2849, D6S1053, D7S3046, D7S1808, D8S1179, D9S2169, D10S1426, D10S2470, D12S391, D17S1294, D17S1299, and D18S51, as required by claims 5 and 19.

Applicants respectfully submit that the Examiner has failed to establish a prima facie case of obviousness because the prior art does not teach all of the claim limitations, there is no motivation to combine the prior art teachings to make the claimed invention, and even if one were motivated to combine the prior art teachings, the art does not provide a reasonable expectation of success. Accordingly, Applicants respectfully request that the rejection be withdrawn.

As the application is now in condition for allowance, Applicants respectfully request withdrawal of the rejections and allowance of the claims. This response is being filed within six months of the mailing date of the Office Action, and is accompanied by a petition for a three-month extension of time and check number 47501 in the amount of $930.00 for the extension of time fee. No other fees are believed due in connection with this submission. If any additional fees are due, please charge such fee to Deposit Account No. 50-0842.

Applicants invite the Examiner to contact the undersigned should he require further clarification concerning this response.

Respectfully submitted,

Date:

Jill A. Fahrlander
Reg. No. 42,518

MICHAEL BEST & FRIEDRICH LLP
One South Pinckney Street
P.O. Box 1806
Madison, Wisconsin 53701-1806
Phone: (608)257-3501

MARKED UP VERSION SHOWING CLEARLY THE CHANGES

22. The method of claim 16, wherein the set of at least [two]three microsatellite loci is a set of at least nine microsatellite loci, comprising: BAT-25, Bat-26, MONO-15, D1S5118, D3S2432, D7S1808, D7S3070, D7S3046, and D10S1426.